Buffer capacity of food components influences the acid tolerance response in Salmonella Typhimurium during simulated gastric passage

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P37: Silver enhances the bactericidal effect of colistin on planktonic *Pseudomonas aeroginosa*

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There is a growing need to optimize antimicrobial therapies given the rising incidence of antibiotic resistant pathogens. Silver has a long-standing history as an antimicrobial agent but still its mechanism of action remains unclear. It has recently been shown that silver may potentiate the activity of antibiotics against Gram-negative bacteria in different metabolic states, as well as to restore antibiotic susceptibility to resistant bacterial strains and also to sensitize Gram-negative bacteria to Gram-positive-specific antibiotics. We investigated whether silver potentiates the effect of colistin as a bactericidal lipopeptide antibiotic active against Gram-negative bacteria. We assumed that silver´s ability to compromise membrane integrity could help cationic colistin to destabilize the membrane. We treated *Pseudomonas aeroginosa* with $AgNO^{3-}$ at 0, 15, 30, and 60µm together with colistin 0, 0.25, 0.5, 1 and 2 µg/ml, respectively, for up to 3 hours to see whether silver cations have a synergistic effect on colistin activity. We applied propidium iodide (PI) as an indicator for both killed bacteria and disintegrated membrane. Cells were analyzed by flow cytometry. The preliminary data suggest that silver could significantly enhance bactericidal effect of colistin, but the results remain to be confirmed and to be supported by counting of colony formation.

P38: Methanotrophs assisted bentazone degradation

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Groundwater, source of drinking water for many countries is increasingly threatened by pesticides contamination, including bentazone, a persistent thiadiazine herbicide. Anaerobic groundwater often contains methane, which is oxidized by methane-oxidizing bacteria (MOB) upon groundwater aeration in rapid sand filter, a common technology for drinking water production. These bacteria have known cometabolic degradation properties against some organic contaminants. Our goal was to test whether MOBs enriched from rapid sand filters can cometabolically degrade bentazone. Hence, we used bioreactors, fed with drinking water and methane, inoculated with material from rapid sand filters rich in methanotrophs, to grow enriched methane-oxidizing biofilms. Batch assays including biomass in drinking water, ^{14}C carbonyl-labeled bentazone with and without methane were investigated (triplicates). Results showed rapid removal; after 48 hours an average of 91% of the initially added bentazone was still detected in autoclaved controls, while 3.7% was detected in microcosms with methane and 6.6% in microcosms without methane. Bentazone removal was evident; the ratio of bentazone/methane affecting its removal rate, although the observed degradation pattern was not a typical cometabolic process. Conclusively, two removal processes were detected depending on the compounds concentrations while the enriched culture's potential in pesticides degradation should be further investigated.

P39: Number and species of parasites not being *Giardia lamblia* or *Cryptosporidium hominis / Cryptosporidium parvum* in patients with diarrhoea

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We are replacing microscopy with species-specific PCR for *Giardia lamblia, C.hominis/ C.parvum* and *E.histolytica* for detection of agents of diarrhoea in patients. Concerned that one may miss true causes of diseases, we decided to change diagnostic approach and establish the number and species of parasites identified by microscopy other than those detected by the PCR assays. Data from a six-year period were extracted from our electronic laboratory information system. Thus, 52,250 stool samples from 21,010 individuals were analysed by microscopy from 1/10-2005 to 1/10-2014, and 2759 stool samples from 1353 patients were positive for parasites.

Giardia lamblia was detected in 500 stool samples from 237 patients, C.*hominis/parvum* in 78 stool samples from 41 patients and *E.histolytica/dispar* in 159 stool samples from 62 patients. We have previously found that parasites reported as *E.histolytica* are almost entirely the non-pathogen *E.dispar*. The only diarrhoea-causing parasite found by microscopy which was not included in the PCR assays was *Cyclospora cayatanensis,* which was detected in 12 patients. Assuming better sensitivity of PCR than microscopy for detection of *G.lamblia* and *C.hominis/parvum*, we find replacement of microscopy with PCR justified. Due to the potential severity of amoebic infections, we find inclusion of PCR for *E.histolytica* relevant. Microscopy examination of faeces is indicated on relevant suspicion of infections with parasites not causing diarrhoea.

P40: Buffer capacity of food components influences the acid tolerance response in *Salmonella* Typhimurium during simulated gastric passage

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Food composition, buffer capacity, and fat and protein content have been shown to effect the gastric acid survival of pathogens (Waterman & Small 1998). In this study, simple food-model substances with different buffer capacities were investigated for their ability to support survival of stationary phase *Salmonella* Typhimurium during simulated gastric acid passage. We used a computer-controlled fermentor to employ pH changes in synthetic gastric fluid, mimicking the dynamic pH during gastric passage. In order to minimise variation, *Salmonella enterica* serovar Typhimurium was contained in dialysis tubes, enabling simultaneous testing of biological triplicates under varying conditions. Surprisingly, we found that less buffered media provided higher protection of Salmonella, compared to media with high buffer capacity. By investigating the relative gene expression of *rpo*S and *omp*R encoding for two major stationary phase ATR regulators, we found an approx. four-fold increase in expression of *omp*R and an approx. three-fold increase of *rpo*S in saline and buffered saline, respectively, after 15 min of gastric acid challenge. The relative expression of these genes, were significantly lower in Brain Heart Infusion Broth having a higher buffer capacity. We suggest this to be associated with a varying ability of *Salmonella* Typhimurium to mount a stationary phase acid tolerance response (ATR) depending on the buffer capacity of the food vehicle.

P41: Gliadin affects glucose homeostasis and intestinal metagenome in C57GL/6 mice fed a high-fat diet

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Dietary gluten and its component gliadin are well-known environmental triggers of celiac disease and important actors in type-1 diabetes, and are reported to induce alterations in the intestinal microbiota. However, research on the impact of gluten on type-2 diabetes in non-celiac subjects is more limited. The aim of this study was to investigate the effect of gliadin on glucose homeostasis and intestinal ecology in the mouse. Forty male C57BL/6 mice were fed a high-fat diet containing either 4% gliadin or no gliadin for 22 weeks. Gliadin consumption significantly increased the HbA1c level over time, with a borderline significance of higher HOMA-IR (homeostasis model assessment of insulin resistance) after 22 weeks. Sequencing of the V3 region of the bacterial 16S rRNA genes showed that gliadin changed the abundance of 81 bacterial taxa, separating the intestinal microbial profile of the gliadin consuming mice from the control mice in the principal coordinate analysis (PCoA) of weighted UniFrac distance. No difference was found in body weight gain, feed consumption or circulating cytokines (IL-1β, IL-6, IFN-γ, TNF-α and IL-10). Our study is the first to show that gliadin as part of a defined synthetic feed exacerbates the glycaemia and alters the intestinal microbiota composition. Comprehensive analyses of the profile of specific immune cells, metabolites and intestinal permeability are in progress to elucidate the mechanism behind the observed effects.